Expression of Inducible Nitric Oxide Synthase After Endothelial Denudation of the Rat Carotid Artery

Role of Platelets

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Abstract—There is functional evidence suggesting that endothelial denudation stimulates inducible nitric oxide synthase (iNOS) activity in the vascular wall. In vitro studies have shown that iNOS expression in smooth muscle cells is reduced by endothelial cells. In the present study we have analyzed the time course of iNOS protein expression in the arterial wall after in vivo deendothelialization. Endothelial denudation was performed in the left carotid artery of Wistar rats, and the right carotid artery was used as control. Whereas iNOS protein was weakly expressed 6, 24, and 48 hours after endothelial denudation, a marked iNOS expression was found 7, 14, and 30 days after vascular damage. Because platelet adhesion and aggregation occur early after endothelial damage, we studied the role of activated platelets in the negative modulation of iNOS protein expression during the first 2 days after endothelial denudation. Early after in vivo endothelial injury, platelet-depleted rats showed a marked iNOS protein expression in the vascular wall. Similar results were obtained by blocking the platelet glycoprotein (GP) IIb/IIIa. Although iNOS protein is present in the arterial wall several days after endothelial denudation, early after arterial wall injury iNOS protein is weakly expressed. Platelets play a crucial role in preventing iNOS protein expression early after endothelial damage, an effect that can be avoided with GP IIb/IIIa blockers. Although iNOS protein was weakly expressed in vivo in the rat carotid artery wall 6, 24, and 48 hours after balloon endothelial denudation, a marked iNOS expression was found 7, 14, and 30 days after arterial damage. iNOS expression could be increased early after endothelial injury by removing circulating platelets and by an antibody against the GP IIb/IIIa. In conclusion, platelets prevent iNOS protein expression early after endothelial balloon damage, an effect that can be avoided with GP IIb/IIIa blocking agents. (Circ Res. 1998;83:1080-1087.)

Key Words: angioplasty ■ endothelium-derived factor ■ nitric oxide ■ nitric oxide synthase ■ platelet ■ glycoprotein IIb/IIIa
tion, we have specifically studied the relation of platelets with iNOS expression in the vessel wall.

Materials and Methods

In Vivo Arterial Injury and Tissue Preparation

Male Wistar rats (average weight, 300–50 g; 4 to 6 months old) were used in the present study, approved by the Fundación Jiménez Díaz Committee for Animal Research. All surgical procedures were performed under general anesthesia by an intraperitoneal injection of ketamine (6 mg/100 g body weight [bw]) and chlorhydrate 2-(2,6-xylidine)-5,6 dihydro-4 H-1, 3 thiazene (23 mg/100 g bw). After dissection of the internal and external left carotid bifurcation, a small artery was perfused with paraformaldehyde (10% in 0.1 M sodium cacodylate, pH 7.2). A 2F Fogarty balloon catheter (Baxter Edwards) was inserted. The balloon catheter was advanced into the aorta (≈2 cm below the carotid bifurcation). The balloon was inflated with 0.2 mL of air and then withdrawn to the entry point. The entire procedure was performed 3 times and the external carotid artery was ligated after the removal of the catheter. To prevent endotoxemia, 2 days before producing the endothelial denudation, the rats were treated with 1.17 g/L amoxycillin and 0.29 g/L clavulanic acid. This treatment was continued for 3 days after surgery.

The balloon-injured arteries were collected at 6, 24, and 48 hours and 7, 14, and 30 days. In all experiments, the contralateral right carotid artery was used as control. At the indicated times, the rats were anesthetized and exsanguinated. The remaining blood was washed out by perfusing 100 mL isotonic saline through the abdominal aorta at a pressure of 100 mm Hg. Immediately after these maneuvers, both carotid arteries were removed and quick frozen in liquid nitrogen for iNOS protein determination.

Determination of iNOS Protein Expression

The iNOS protein was analyzed by Western blot as described elsewhere. The frozen carotid arteries were pulverized and solubilized in Laemmli buffer containing 2-mercaptoethanol. Proteins were separated in denaturing SDS/10% polyacrylamide gels. Equal amounts of proteins (15 μg/lane) were loaded. To verify that equal amounts of proteins had been loaded in the gel, a parallel gel with identical samples was run and stained with Coomassie to compare the intensities of the protein bands. The separated proteins were then blotted into nitrocellulose (Immobilon-P, Millipore Corp). Blots were blocked overnight at 4°C with 5% nonfat dry milk in TBS-T (20 mM Tris-HCl, 137 mM NaCl, 0.1% Tween 20). Western blot analysis was performed with a monoclonal antibody against iNOS protein (Transduction Laboratories). Blots were incubated at room temperature with the first antibody (1:250) during 1 hour and, after extensive washing, with the second antibody (horseradish peroxidase–conjugated anti-mouse immunoglobulin antibody) at a dilution of 1:1500, for another hour. Specific iNOS protein was detected by enhanced chemiluminescence (ECL, Amersham) and evaluated by densitometry (Molecular Dynamics). Prestained protein markers (Sigma Chemical) were used for molecular mass determination.

To assess the specificity of the iNOS monoclonal antibody, 2 different approaches were used. First, we analyzed the cross-reactivity of the antibody against the endothelial NOS isoform with a homogenate of rat endothelial cells. Cultures of rat endothelial cells were prepared as described by McGuire et al. Specific immunofluorescence staining for factor VIII-related antigen was used previously to identify rat aorta endothelial cells. The second maneuver was to confirm the antibody ability to recognize the iNOS isoform expressed in homogenates of Escherichia coli lipopolysaccharide (LPS)-treated rat macrophages.

Immunohistochemistry

Carotid arteries were collected from different groups of rats. Rats were perfused with 50 mL of fixative solutions containing 4% paraformaldehyde in serum saline (1:1 vol/vol). Injured carotid arteries were removed and dehydrated with sequentially increasing concentrations of ethanol followed by xylene and embedded in paraffin wax. Paraffin blocks were sectioned at 4 μm on a standard rotary microtome (Leitz 1512) and the sections were recovered from a water bath on acid-alcohol–cleaned slides. Sections were stained with hematoxylin and eosin with an automated staining system.

The sections were further incubated with PBS containing 3% normal goat serum (ICN Biochemicals Ltd) and 0.1% Triton X-100 for 30 minutes and subsequently with polyclonal antibody against iNOS protein (Calbiochem) or monoclonal antibodies against monocytes/macrophages CD68 antigen (clone KP51) and α-smooth muscle actin (clone IAA4, Sigma Chemical) at a dilution of 1:500 for 1 hour. The sections were washed in PBS containing 0.05% BSA, then incubated with biotinylated antiserum to goat IgG (1:300 dilution, Vector Laboratories Ltd) for 1 hour and in a solution of peroxidase-linked avidin-biotin complex (ABC) (ABC kit, Vector Laboratories Ltd) for another hour as previously reported. To reveal the peroxidase activity, the nickel-enhanced diaminobenzidine procedure was used.

Production of Rat Antiplatelet-Enriched Serum

Antiplatelet-enriched serum was obtained as described. Pure isolated platelets obtained from control Wistar rats were homogenized in Freund’s complete adjuvant and injected subcutaneously in rabbits. Rabbit plasma was collected by plasmapheresis 10 days after a second immunization. Nonimmune serum was obtained from nonimmunized rabbits and served as control (control IgG).

Wistar rats were injected with the antiplatelet (APab)–enriched serum or control serum IP (30 mg/100 g bw) 24 hours before ballooning. This dose of APab resulted in severe thrombocytopenia with platelet counts <10,000/μm² within 24 hours, representing an 85% reduction (P<0.01) relative to platelet counts in control IgG serum-treated rats. The injured carotid artery was collected 6, 24, and 48 hours after ballooning.

Determination of Tumor Necrosis Factor-α (TNF-α) Plasma Levels

We measured TNF-α levels in the plasma obtained from both APab- and IgG-treated rats 24 hours after the endothelial denudation. Peripheral blood was collected into 10% (vol/vol) acid-citrate-dextrose and the plasma was isolated after centrifugation at 2500 rpm, 15 min, 4°C. We determined TNF-α plasma levels with a commercial ELISA kit (Chromogenix). The intra- and interassay variabilities of the ELISA kit were 1.1% and 4.7%, respectively.

iNOS Protein Expression After Blockade of Glycoprotein IIb/IIIa Receptors

Rats received c7E3 Fab (abciximab; Reopro, Eli Lilly, and Centocor), the Fab fragment of the chimeric human-murine monoclonal antibody c7E3. This agent binds to the platelet glycoprotein (GP) IIb/IIIa receptor. We administered c7E3 Fab as an intravenous bolus (500 μg/kg bw) 10 minutes before deendothelialization, followed by a continuous infusion of 10 μg/kg bw for 60 minutes after the endothelial denudation. Rats treated with a nonspecific IgG administered in the same way and doses as c7E3 Fab served as control. Damaged carotid arteries were collected 24 hours after endothelial denudation.

Ex Vivo Platelet Aggregation

We evaluated platelet aggregation in response to ADP to assess the degree of receptor blockade induced by the treatment with c7E3 Fab. Immediately after completing the GP IIb/IIIa antibody 60-minute infusion, blood was collected and plasma-rich platelet (PRP) obtained as already described. We adjusted the platelet number with platelet-poor plasma obtained from the same rat to 2.5×10⁶ cells/mL. Platelet activation was measured by an aggregometer (Chronolog, 2 channels) as described elsewhere. Previously, a platelet-poor sample was used as control for 100% light transmission. PRP (500 μL) was incubated at 37°C in the aggregometer with continuous stirring (1000 rpm) and then stimulated with ADP (10⁻⁶ mol/L).
For standardization of measurements, only the value obtained by turbidimetry at 3 minutes was used for the calculations.

Platelet Labeling
A group of 6 Wistar rats were used as blood donors to obtain isolated platelets which were radiolabeled with $^{51}$Cr (Amersham Life Science Ltd). PRP was obtained as mentioned above and then incubated with 400 $\mu$L $^{51}$Cr for 30 minutes. The platelet suspension was then centrifuged to remove the unbound $^{51}$Cr, resuspended in plasma, and reinjected into new Wistar rats. Radiolabeled platelets were allowed to circulate for 1 hour before endothelial denudation. The platelet count per milliliter of blood remained similar before and at the end of the experiments in all groups. Twenty-four hours after the endothelial denudation procedure, the carotid arteries were removed and washed several times with saline. The contralateral carotid artery was also obtained as control.

The artery segments were weighted and then counted in a gamma counter. The radioactivity in each segment was corrected for the percentage of free $^{51}$Cr in plasma (unbound to platelets). With knowledge of the whole blood platelet count, the number of platelets deposited on the carotid artery was calculated with the formula: No deposited platelets = $\frac{[^{51}$Cr counts per minute [cpm] in the carotid artery]$\times$[no platelets/mL blood]}{[$^{51}$Cr cpm/mL blood]}.$ The number of platelets per weight unit was then obtained by dividing the number of deposited platelets by the weight of the carotid artery.

Relation Between Platelet-Derived Growth Factor and iNOS Expression
Because platelet-derived growth factor (PDGF) has been shown to inhibit iNOS protein expression in cultured smooth muscle cells, we tested the involvement of this growth factor in iNOS expression after endothelial denudation. For this purpose, the rats received oral treatment with a competitive PDGF-receptor antagonist, trapidil (triazolopyrimidine, UCB Pharma). Trapidil was added to drinking water (20 mg/kg bw) from 2 days before ballooning and until the rats were euthanized (24 hours after endothelial denudation). A control group of rats receiving water served as controls. In both groups of rats, the damaged carotid arteries were also collected 24 hours after endothelial denudation.

Statistical Methods
Results are expressed as mean±SEM. Unless otherwise stated, each value corresponds to carotid segments isolated from a minimum of 6 different rats. To determine the statistical significance of our results, we performed an ANOVA with Bonferroni’s correction for multiple comparisons or a Student’s t test (paired or unpaired). A $P$ value $<0.05$ was considered statistically significant.
Results

iNOS Protein Expression After In Vivo Endothelial Denudation

Modifications of the levels of iNOS protein expression were detected by Western blot. As shown in Figure 1A and 1B, iNOS protein expression was well evident in the injured artery 7 to 30 days after endothelial denudation. Conversely, 6 to 48 hours after the endothelial damage, iNOS protein was weakly expressed (Figure 1A and 1B). However, the level of iNOS protein expression observed early after endothelial denudation (6 to 48 hours) was higher than that observed in the contralateral endothelialized control arterial segment (Figure 2A and 2B). Moreover, in the uninjured contralateral artery, the expression of iNOS protein was undetectable at all tested times (data not shown).

The monoclonal antibody used did not cross-react with the endothelial NOS isoform because an iNOS protein band was undetectable in a homogenate of rat aortic endothelial cells (Figure 2C). We previously reported similar findings by using homogenates of bovine aortic endothelial cells.11,12 The monoclonal antibody used in our experiments specifically recognized the iNOS isoform (130 kDa) obtained from homogenates of LPS-treated rat macrophages (Figure 2C).

The histological examination also revealed that during the early times after endothelial damage, iNOS protein expression was weakly expressed (Figure 3A). Uninjured contralateral carotid arteries showed no detectable iNOS signal (data not shown); iNOS protein was markedly present several days after the endothelial injury (Figure 3B). Control immunostaining in which the primary antibody was replaced with normal rabbit serum produced no positive signal (data not shown).

Immunolocalization of iNOS protein demonstrated that the cells which expressed iNOS protein were also positively stained by α-actin indicating the presence of vascular smooth muscle cells (Figure 3C). Furthermore, the presence of CD68 antigen, a monocyte/macrophage marker,20 in the injured vessels was negative 7 days after the endothelial denudation (Figure 3D) a time in which iNOS protein was markedly expressed. Moreover, immunostaining for CD 68 antigen was negative at all the experimental times (data not shown), which was confirmed by hematoxylin and eosin staining.

Figure 3. Cross sections of endothelial denuded carotid arteries. The expression of iNOS protein was detected by a polyclonal antibody against iNOS in paraffin sections obtained 24 hours (A) and 7 days (B) after the arterial injury. iNOS is indicated by arrows. C, Shows positive immunostaining for α-actin 7 days after the endothelial denudation of rat carotid arteries. D, Shows the absence of CD68 antigen, a monocyte/macrophage marker, 7 days after endothelial damage. Bar=25 μm.
iNOS Protein Expression in Thrombocytopenic Rats After In Vivo Endothelial Denudation

Because several studies have previously established that platelet activation occurs immediately after endothelial denudation, we examined if platelets could be responsible for the slight expression of iNOS protein observed in the first hours after endothelial injury. For this purpose, the rats were treated with APab-enriched serum 24 hours before ballooning. At 6, 24, and 48 hours and at 7 and 30 days after endothelial denudation, the left carotid artery was removed to determine iNOS protein expression.

Endothelial-denuded carotid arteries of APab-treated rats showed a marked iNOS protein expression 6, 24, and 48 hours after balloon injury (Figure 4A and 4B). In contrast, at each corresponding time, the carotid arteries obtained from the control IgG-serum–treated rats showed a weak and lesser expression of iNOS protein (Figure 4A and 4B).

Because platelets adhering to the injured vessel could provide a defense barrier to limit the early infiltration of iNOS-expressing leukocytes, we localized iNOS expression by immunohistochemistry in the APab-treated rats. iNOS protein was present in a pattern essentially identical with that observed in the rats with platelets (Figures 3B and 5A).

Furthermore, thrombocytopenic rats showed no detectable CD68 staining indicating the absence of monocyte/macrophages in the injured vessel (Figure 5B).

In the APab-treated rats, iNOS expression was more evident 24 and 48 hours than 6 hours after endothelial denudation (Figure 4A and 4B). The specificity of APab was demonstrated by its inability to reduce the counts of circulating leukocytes, because the latter were similar in APab-treated and in IgG-treated animals (leukocyte numbers \( \times 10^7/\text{mm}^3 \); IgG-treated rats, 1.38±0.5; APab-treated rats, 1.43±3; \( n=6 \) different animals, pNS). In addition, circulating TNF-\( \alpha \) plasma levels were equal in both APab-treated and IgG-treated deendothelialized rats (TNF-\( \alpha \) (pg/mL): IgG-treated rats, 11±3; APab-treated rats, 13±4; \( n=6 \) different animals, pNS).

In further experiments, we analyzed if platelet depletion modified iNOS expression 7 and 30 days after injury. As shown in Figure 4A and 4B, 7 and 30 days after injury iNOS protein expression was slightly, although significantly, greater in APab-treated than in IgG-treated rats.

iNOS Protein Expression and Inhibition of Platelet Activation After Endothelial Denudation

Blockade of the GP IIb/IIIa receptor with c7E3 Fab increased iNOS protein expression early after vessel injury (Figure 6A and 6B). In contrast, a nonspecific IgG treatment did not modify iNOS protein expression 24 hours after endothelial denudation compared with the expression observed in water-treated rats (placebo group in Figure 6A and 6B).

The anti-aggregating platelet effect caused by the blockade of GP IIb/IIIa by c7E3 Fab treatment was assessed by...
aggregometry. c7E3 Fab treatment completely blocked platelet aggregation in response to ADP (% light transmission: IgG-treated rats, 50±2; c7E3-treated rats, 7±3; n=6, P<0.05).

We further analyzed whether c7E3 Fab reduced platelet adhesion to the injured site. Platelet deposition on uninjured segments was <0.25×10⁶/mg. A marked increase in platelet deposition was detected in the injured carotid artery of IgG-treated rats 24 hours after endothelial denudation (increase in platelet deposition 40±4×10⁶ platelets/mg; n=5 different animals; P<0.01). Blockade of GP IIb/IIIa produced a significant reduction of platelet deposition on the injured carotid artery (22±3×10⁶ platelets/mg; n=5 different animals; P<0.05 with respect to IgG-treated rats). However, the number of platelets deposited on the injured artery of c7E3 Fab–treated rats was significantly greater than on the endothelialized contralateral uninjured artery (P<0.01).

Finally, we determined the involvement of PDGF in the effect of platelets on iNOS protein expression. Treatment with trapidil, a PDGF receptor antagonist, increased iNOS protein expression 24 hours after endothelial denudation (Figure 6A and 6B). However, the level of iNOS expression achieved with trapidil was systematically of a significantly lesser magnitude than that obtained after blocking GP IIb/IIIa receptors (Figure 6A and 6B). An increased concentration of trapidil (40 mg/kg bw) failed to demonstrate a greater iNOS protein expression than that obtained with 20 mg/kg bw trapidil (data not shown).

Discussion

In the present study we have shown that iNOS protein is present in the arterial wall several days after endothelial denudation. Early after arterial wall injury, iNOS protein expression is very weak. Platelets play a crucial role in preventing iNOS expression early after endothelial damage, and this negative modulatory effect can be prevented by GP IIb/IIIa–blocking drugs.

Previous in vitro studies showed that early after endothelial denudation vascular reactivity to vasoconstrictor agents is decreased through a mechanism that is blocked by NO synthesis inhibitors. In vivo coculture studies performed in our laboratory also showed that endothelial cells downregulate iNOS protein expression in smooth muscle cells. Hassan et al performed in vivo experiments suggesting that iNOS expression was rapidly induced in the arterial wall after endothelial injury. In addition, the injured smooth muscle cells could release cytokines that are able to stimulate iNOS protein expression. Therefore, early after the vessel injury one should expect to find iNOS protein expression in the carotid artery wall. Our observation that iNOS expression was weak during the first 48 hours after endothelial denudation was thus surprising.

The weak level of iNOS protein expression that we found early after endothelial denudation is insufficient to explain the aforementioned functional effects attributed to the iNOS activity. The appearance of iNOS expression and activity in the previously quoted in vitro and in vivo studies could be caused by the presence of endotoxemia after surgery or by endotoxins contained in the incubation medium because the authors in these studies did not report on the use of antibacterial treatment. In the present study, the rats underwent prophylactic treatment with antibiotics; therefore, the presence of iNOS expression related to endotoxemia should be discarded.

The precise identification of the cells expressing iNOS protein in the wall of the injured carotid artery demonstrated positive iNOS immunostaining in actin-positive vascular smooth muscle cells and discarded the presence of monocytes/macrophages in the damaged rat carotid artery. Schwartz et al also reported the absence of infiltrating leukocytes in this same animal model. Because a high expression of iNOS protein occurred several days after endothelial denudation, the present study asked why only a weak iNOS protein expression was observed between 6 and 48 hours after the vascular damage.

Disruption of the endothelial continuity of blood vessels results in rapid platelet activation. Thus, in the second part of the present study we analyzed whether platelets could be involved in the weak expression of iNOS protein early after carotid injury.

To assess this hypothesis we followed several experimental steps. First, we tested if platelets regulate in vivo iNOS protein expression by performing the endothelial denudation in thrombocytopenic rats. These rats showed a marked expression of iNOS protein early after vascular wall damage and also slightly but significantly enhanced the later expression. These results could suggest that platelets inhibited the early expression of iNOS protein which could also modulate...
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the later presence of this protein in the injured vessel. Second, we investigated if the APab treatment could affect blood cellular elements other than platelets and specifically, if leukocytes could be stimulated and release more cytokines favoring iNOS expression in thrombocytopenic rats. This hypothesis was discarded because circulating levels of TNF-α, a cytokine released from leukocytes were not different in APab-treated and in nonspecific IgG-treated rats. Furthermore, leukocyte counts were not modified by APab treatment. It is noteworthy that platelets were not a physical barrier to limit the early infiltration of iNOS-expressing monocytes/macrophages as demonstrated by the absence of these cells on the injured carotid arteries of thrombocytopenic rats.

Platelet activation results in exposure and activation of GP IIb/IIIa receptors on the platelet surface. GP IIb/IIIa receptors are pivotal mediators of platelet aggregation and are involved in the adhesion to the injured vessel. Our third step was then to examine if the blockade of GP IIb/IIIa platelet receptors favored iNOS protein expression early after endothelial denudation. We found that the blockade of GP IIb/IIIa receptors by c7E3 Fab stimulated iNOS protein expression in the endothelial-denuded vascular wall. The ability of c7E3 Fab to block rat platelet activation was demonstrated in the ex vivo platelet aggregation experiments. These results strongly support the direct implication of activated platelets on the inhibition of iNOS protein expression immediately after endothelial denudation.

The blockade of GP IIb/IIIa augmented iNOS protein expression 24 hours after injury to a significantly lesser degree than that observed after platelet depletion. GP IIb/IIIa reduced but did not abolish platelet adhesion to the injured vessel. This could mean that platelet adhesion is important for the platelet effect on iNOS expression. In this regard, the processes by which platelets are recruited at the site of vascular injury are modulated by several proteins expressed on the platelet surface and not only by GP IIb/IIIa activation.

GP IIb/IIIa blockade prevents thrombogenesis and reduces neo-intima formation after vascular damage. Furthermore, Fingerle et al. have demonstrated that depletion of circulating platelets reduced the size of the intimal lesion after balloon injury, suggesting that activated platelets play a relevant role in the neo-intimal formation. Further experiments are needed to define the importance of iNOS activity in the above-mentioned prevention of neo-intima formation related to platelet depletion and particularly to GP IIb/IIIa receptor blockade.

Binding of platelets to the injured vessel wall results in the release by platelets of a number of agents including PDGF, an in vitro inhibitor of iNOS expression in cultured smooth muscle cells. We found that trapidil, a PDGF receptor antagonist, increased iNOS protein expression early after endothelial denudation. However, the increase in iNOS protein expression related to trapidil was of a lesser magnitude than that observed in c3E7 Fab–treated rats, suggesting that other platelet-derived agents could be involved in the modulation of iNOS expression. Other factors released from activated platelets such as transforming growth factor-β are known to inhibit iNOS expression by smooth muscle cells in culture; therefore, further studies are needed to unravel this point. The finding that iNOS protein expression was well-evident in the injured arterial wall 7 to 30 days after endothelial denudation could be explained by the fact that platelets disappear from the arterial surface a few days after the damage.

The beneficial or deleterious role of iNOS activity after arterial endothelial denudation remains to be studied. In addition to its effects on platelet activation, NO inhibits smooth muscle cell proliferation and the synthesis of extracellular matrix proteins could prevent neointimal formation. Fukumoto et al. have reported that NO generated by iNOS exerts on inhibitory effect against the cytokine-induced proliferative changes of the coronary artery in vivo. On the other hand, NO is a cytotoxic molecule for endothelial cells and also inhibits their growth. The rapid reendothelialization of the injured vessel is a critical phenomenon to block neointimal formation. However, continuous generation of NO by iNOS was recently associated with the impairment of the NO system in endothelial cells which was speculated to protect the vascular wall from excessive amounts of NO.

In conclusion, balloon-injured rat carotid arteries markedly expressed iNOS protein several days after the intervention. Early after the arterial injury, platelets seem to play a crucial role in the negative modulation of iNOS protein expression in the damaged vascular wall. Further studies are needed to elucidate whether the protective effects of antiplatelet therapy in the acute coronary syndromes are partly due to their ability to increase iNOS protein expression early after endothelial disruption.

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